AD-A056 402 GEORGIA UNIV ATHENS COLL OF VETERINARY MEDICINE F/G 6/20
IMPROVED THERAPY OF LEISHMANIASIS BY ENCAPSULATION OF ANTIMONIA--ETC(U)
JUN 78 C R ALVING, E A STECK, W L HANSON DAMD17-75-C-5011

UNCLASSIFIED NL

| OF |
AD
A056402

END
DATE
FILMED
8-78
DDC

*ALVING, STECK and HANSON

LEVEL II

(15) DAMD17-75-C-5017

DDC
RECEIVED
JUL 10 1978
D

(6) IMPROVED THERAPY OF LEISHMANIASIS BY ENCAPSULATION OF ANTIMONIAL DRUG IN BIODEGRADABLE ARTIFICIAL PHOSPHOLIPID VESICLES (LIPOSOMES) (U)

JUN 1978

(10) *CARL R. ALVING, M.D., LTC, MC
EDGAR A. STECK, Ph.D. AND WILLIAM L. HANSON, Ph.D
WALTER REED ARMY INSTITUTE OF RESEARCH,
WASHINGTON, D.C. 20012, AND COLLEGE OF VETERINARY MEDICINE,
THE UNIVERSITY OF GEORGIA, ATHENS, GA 30602

(11) Jun 78

(12) 8 p.

INTRODUCTION

Leishmania, which are hemoflagellate protozoa, are important pathogenic intracellular parasites which cause diseases resulting in cutaneous, mucocutaneous, or visceral (kala azar) manifestations. The parasites reside chronically in phagocytes of the reticulo-endothelial system (1-4). At least 12 million people are infected with various forms of Leishmania (4). Leishmaniasis is highly infectious and represents a significant potential military health problem. Leishmania donovani was first demonstrated in smears taken post-mortem from the spleen of an English soldier in 1900 in India (2). Epidemics have occurred among soldiers fighting in the forest, as in Paraguay in the Gran Chaco war (3). In recent years, armed forces that have operated in endemic areas, such as Colombian Army, the Israeli Army in the Sinai, and the U.S. Army in Panama, have had high attack rates in certain units. The disease is endemic in the Middle East, Africa, India, China, Asian USSR, Central and South America, and other tropical and subtropical regions throughout the world. The illness may be severe, lingering, and may be recurrent despite therapy with antimonial compounds, the drugs of choice (1,4).

Treatment of leishmaniasis is hampered, and doses are limited, by the serious toxicities of antimonials (1). We have developed a novel approach to treatment of leishmaniasis which takes advantage of the localization of the organism in phagocytic cells. Our technique consists of injection of liposomes containing antimonial drugs. Liposomes are artificial, biodegradable membranes comprised of

lipids, including phospholipids and other lipids that can be obtained from natural or synthetic sources (5,6). As shown schematically in Fig. 1, the membranes are in the form of closed concentric spheres

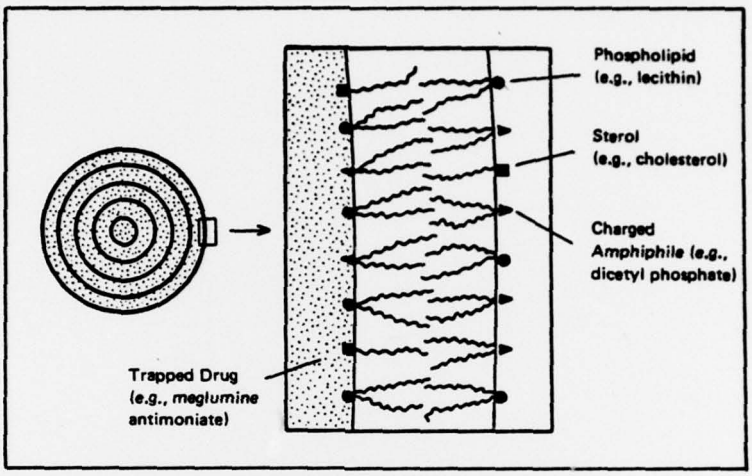


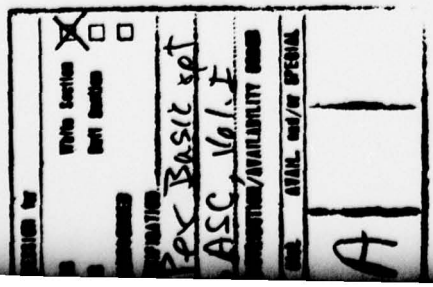
Figure 1. Schematic illustration of a liposome

separated by aqueous interspaces. Various drugs can be trapped in the internal aqueous regions of the particles (7-10). Upon intravenous injection the liposomes automatically and rapidly (within minutes) "home" to the same cells that contain the Leishmania, namely the macrophages in the reticuloendothelial system (7-10). The drug is gradually released in a high localized dose in the vicinity of the parasite. In this paper we describe the effect of liposome-encapsulated antimonial drug on the treatment of experimental leishmaniasis in hamsters.

MATERIALS AND METHODS

Testing of suppressive effects of drugs in an experimental infection was performed by a slight modification of previously published methods (11,12). Yound 50-70 g golden hamsters (Mesocricetus auratus) were injected intracardially with the Khartoum strain of Leishmania donovani. Each injection contained 10^7 amastigotes obtained from spleens of donor hamsters.

After either 3 days, 10 days, or 17 days post-inoculation of



parasites, test drugs in three different dosage levels were administered intracardially daily, under "blind" experimental conditions, for four consecutive days. Intracardial injections were used because intravenous administration is not practical in hamsters, and intramuscular or intraperitoneal routes were not effective. Eight or nine animals were used for each experimental group. One day after the last treatment injection, all of the animals of each group were sacrificed. The livers were removed, and the total numbers of parasites per liver were determined from impression smears. Percent of parasite suppression at each dosage level was calculated by comparison to a parallel control group consisting of 6-8 infected, untreated animals that had been injected intracardially with corresponding volumes of normal saline.

Lipids were purchased from the following sources: phosphatidyl choline (Sigma Chemical Co., St. Louis, MO); cholesterol (Calbiochem, La Jolla, CA); dicetyl phosphate (K and K Laboratories, Plainview, NY). Meglumine antimoniate (Glucantime ^{R}) was purchased from Rhodia, Inc., New York, NY. Sodium stibogluconate (Pentostam ^{R}) powder was generously supplied as a gift by Dr. R.A. Neal, Wellcome Foundation Ltd., Beckenham, Kent, England.

Liposomes were prepared from a mixture of dipalmitoyl phosphatidylcholine (Sigma Chemical Co.), cholesterol (Calbiochem), and dicetyl phosphate (K and K Laboratories) in molar ratios of 2/1.5/0.22. The lipids, in chloroform, were dried in a pear-shaped flask on a rotary evaporator, followed by one hour under high vacuum in a desiccator. A small amount of acid-washed 0.5 mm glass beads was added, and followed by addition of a sufficient quantity of either 0.15 M NaCl or 0.308 M Meglumine Antimoniate (obtained from Rhodia, Inc. as Glucantime ^{R}) such that the phosphatidyl choline was 10 mM with respect to the final aqueous dispersion. The liposomes were swollen by shaking for two minutes on a Vortex mixer.

They were washed three times by diluting in 10 volumes of 0.154 M NaCl and centrifuging at 20,200g for 10 min at 22°. The final pellet was suspended with sufficient 0.15 M NaCl so that the phospholipid was about 10-80 mM with respect to the aqueous suspension. An aliquot (0.4 ml) was shaken with 2.1 ml of water and 2.5 ml of chloroform to disrupt the liposomes; the chloroform was washed twice with 2.5 ml of water; and the combined aqueous phases were sent an analytical laboratory (Galbraith Laboratories Inc., Knoxville, TN) for quantitative antimony analysis. The washed liposomes trapped ca. 2-11% of the antimonial drug present in the original swelling solution.

RESULTS

Fig. 2 compares the efficacies of encapsulated and unencapsulated meglumine antimoniate (Glucantime [R]) in suppression of leishmaniasis. It is evident that the encapsulated drug was superior to the unencapsulated drug, or to liposomes alone. Calculations from the data of Fig. 2, based on the amount of drug required to cause 50% suppression, suggested that the liposome-encapsulated drug was approximately 350 times more effective than the drug alone.

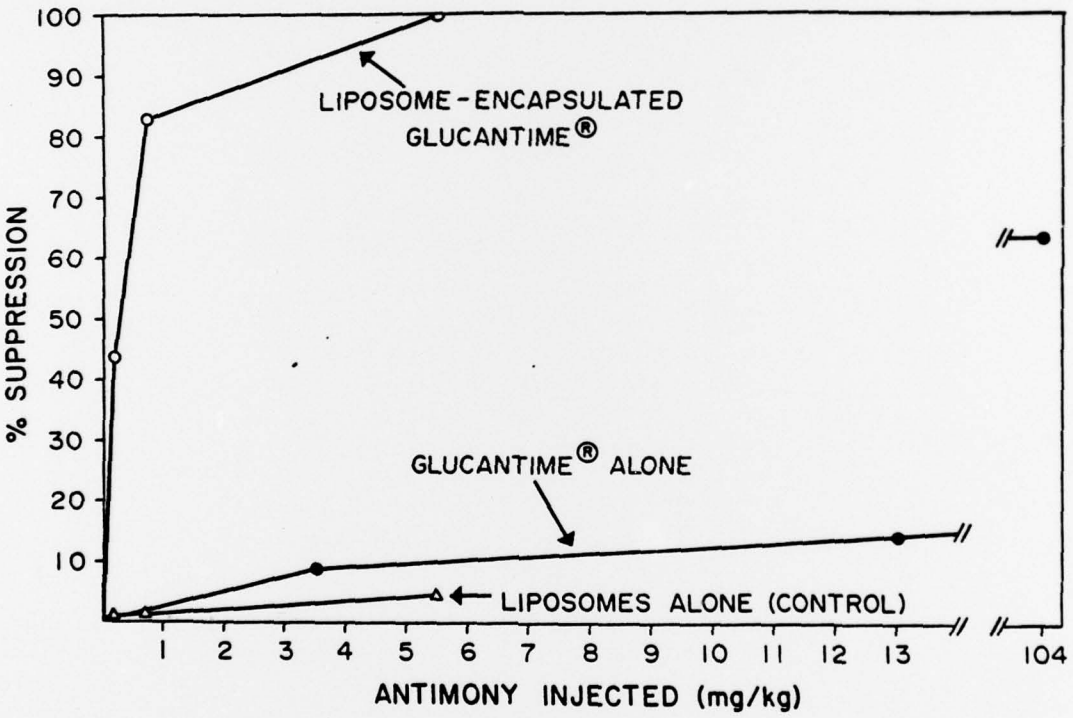


Figure 2.

Suppression of Leishmanial Infection by Liposome-Encapsulated Antimonial Drug. The hamsters had been infected for 10 days prior to treatment, and the indicated doses were administered daily for four consecutive days. In the control the equivalent volumes of liposomes swollen in normal saline were given instead of liposomes swollen in antimonial.

The data of Fig. 2 were obtained with animals whose treatment had been initiated 10 days following infection. Table 1 shows that the length of infection influenced the efficacy of therapy. Infections were compared that were 3, 10 or 17 days in duration before starting therapy. The superior effectiveness of liposome-encapsulated meglumine antimoniate was greater in a long-term (17 day) infection that it was in shorter term (3 or 10 day) infections. It should be noted that in Table 1 more than 100 times as much uncapsulated, compared to encapsulated, drug was used.

TABLE 1. Influence of Length of Infection on Efficacy of Treatment

Treatment Used	Time between infection and start of treatment (days): 3	10	17
	%Suppression		
Liposomes containing Glucantime^{R} (1 mg/kg/day for 4 days)	99.8	82.8	61.3
Glucantime^{R} alone (104 mg/kg/day for 4 days)	99.8	63.7	18

Two different antimonial drugs, meglumine antimoniate (Glucantime ^{R}) and sodium stibogluconate (Pentostam ^{R}) were compared in a long-term (17 day) infection (Table 2). The doses required to produce 50% suppression (SD_{50}) were determined. Under the conditions used, the liposome-encapsulated drugs were enhanced 700-900 times compared to the unencapsulated drugs (Table 2).

DISCUSSION

Prior to the introduction of currently used drugs, mortality in visceral leishmaniasis was higher than 90%; now it is reported at a still high level of 2-5% (3). The only drug available in the United States for treating all forms of leishmaniasis is sodium stibogluconate, and it has the status of an Investigational New Drug (IND) (1, 4). In the present study we demonstrate that encapsulation of sodium

TABLE 2. Relative Efficacies of Two Liposome-Encapsulated, or Unencapsulated, Antimonial Drugs in Treatment of a 17 Day Leishmanial Infection.[a]

Therapeutic Agent	SD_{50}	Enhancement Factor
Glucantime[R]	175	———
Liposome-Encapsulated Glucantime[R]	0.24	729
Pentostam[R]	450	———
Liposome-Encapsulated Pentostam[R]	0.52	865

[a] The SD_{50} is defined as the amount of drug antimony (mg/kg/day for 4 days) required to cause 50% parasite suppression. The enhancement factor is the SD_{50} ratio of liposome-encapsulated drug vs. unencapsulated drug. In this experiment, dimyristoyl, rather than dipalmitoyl, phosphatidyl choline was used.

stibogluconate in liposomes resulted in approximately a 900-fold increased efficacy compared to unencapsulated drug (Table 2). The differences were more marked in long-term "chronic" than in short-term "acute" infections (Table 1). Chronic infections are the types most likely to be encountered in patients at the time of therapy. Meglumine antimoniate is widely used in other countries (1,4), and its effectiveness also was greatly enhanced (more than 700-fold) by encapsulation in liposomes (Table 2).

Antimonial agents are the drugs of choice in leishmaniasis, and in case of treatment failure another toxic drug, amphotericin B, often is used as a last resort (1,4). Antimony belongs to the same periodic group as arsenic, and toxicities, particularly to the heart, kidneys and liver are similar to those of arsenicals (1,13). Although the pentavalent antimonials, such as meglumine antimoniate and sodium stibogluconate, are a great improvement over trivalents, the pentavalents still are highly toxic, particularly in high or prolonged dosage, or in undernourished individuals (1). The doses of antimonials that can be given to patients are limited by potential toxicity, and

the ratio of (effective dose/toxic dose) is not very high. Although we do not have definitive evidence yet, we anticipate that encapsulation of antimonial drug in liposomes, and rapid uptake of liposome by reticuloendothelial cells, will minimize systemic toxic antimonial effects, especially those due to acute cardiomyopathy and toxic nephritis. Toxicity also may be minimal because, based on our animal model, less than 0.15% of an ordinary therapeutic dose may be used for equivalent results. In the doses approved for clinical use (for example, under the conditions of the IND for sodium stibogluconate) treatment failures are common. Because of the greatly enhanced efficacy of liposome-encapsulated drugs, it may be possible to overcome the problem of treatment failure by raising the dosage without increasing the risk of severe toxicity.

SUMMARY

We describe a novel technique for treating leishmaniasis by encapsulation of antimonial drugs in liposomes. The liposomes travel in the bloodstream to the same cells in which the Leishmania organism lives, namely the phagocytes of the reticulothelial system in the liver and spleen. The systemic toxicities of the antimonial agents, which ordinarily are substantial, presumably would be minimized by encapsulation in liposomes and by rapid uptake of liposomes by phagocytes. In an animal model, consisting of experimental visceral leishmaniasis in hamsters, we found that liposome-encapsulated antimonials were 350 - 900 times more effective than unencapsulated drugs in suppressing the infection.

ACKNOWLEDGEMENT

Supported in part by United States Army Medical Research and Development Contract No. DAMD-17-C-5011. The authors are indebted to Mrs. Mary Watson for preparation of the manuscript.

REFERENCES

1. Steck, E.A. The Leishmaniases. In: Progress in Drug Research, Vol. 18, (E. Jucker, Ed.) Birkhäuser Verlag, Basel, pp 290-351, 1974.

2. Faust, E.C., Beaver, P.C., and Jung, R.C. The Leishmania Parasites of Man. In: Animal Agents and Vectors of Human Disease, Lea and Febiger, Philadelphia, pp. 34-64, 1968.

3. Biagi, F. Leishmaniasis-Introduction, Kala Azar, Cutaneous and Mucocutaneous Leishmaniasis. In: Tropical Medicine, 5th ed. (G.W. Hunter, III, J.C. Swartzwelder, and D.F. Clyde, Eds.), W.B. Saunders,

Co., Philadelphia, pp. 411-429, 1976.

4. Mahmoud, A.A.F., and Warren, K.S. Algorithms in The Diagnosis and Management of Exotic Diseases. XXIV Leishmaniases. J. Inf. Dis. 136: 160-163, 1977

5. Bangham, A.D. Membrane Models with Phospholipids. Prog. Biophys. Mol. Biol. 18: 29-95, 1968.

6. Paphadjopoulos, D. Phospholipid Membranes as Experimental Models for Biological Membranes. In: Biological Horizons in Surface Science (L.M. Prince, and D.F. Sears, Eds.) Academic Press, NY, pp. 159-225, 1973.

7. Gregoriadis, G. The Carrier Potential of Liposomes in Biology and Medicine. New Eng. J. Med. 295: 704-710, 765-770, 1976.

8. Tyrrell, D.A., Heath, T.D., Colley, C.M., and Ryman, B.E. New Aspects of Liposomes. Biochim. Biophys. Acta. 457: 259-302, 1976.

9. Poste, G., Papahadjopoulos, D., and Vail, W.J. Lipid Vesicles as Carriers for Introducing Biologically Active Materials into Cells. In: Methods in Cell Biology, Vol. 14 (D.M. Prescott, Ed.), Academic Press, NY, pp. 33-71, 1976.

10. Fendler, J.H., and Romero, A. Liposomes as Drug Carriers. Life Sci. 20: 1109-1120, 1977.

11. Hanson, W.L., Chapman, Jr., W.L., and Kinnamon, K.E. Testing of Drugs for Antileishmanial Activity in Golden Hamsters Infected with Leishmania Donovani. Int. J. Parasitol. 7: 443-337, 1977.

12. Alving, C.R., Steck, E.A., Hanson, W.L., Loizeaux, P.S., Chapman, Jr., W.L., and Waits, V.B. Improved Therapy of Experimental Leishmaniasis by Use of a Liposome-Encapsulated Antimonial Drug. Life Sci. 22, 1978 (In Press).

13. Harvey, S.C. Heavy Metals. In: The Pharmacological Basis of Therapeutics, 5th ed. (L.S. Goodman, and A. Gilman, Eds.) Macmillan, NY, pp. 924-945, 1975.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.